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1 Long-term aquaria study suggests species-specific responses of two cold-water
2 corals to macro- and microplastics exposure

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13

14 ABSTRACT

15 Plastic pollution has been identified as a major threat for coastal marine life and ecosystems.

16 Here, we test if the feeding behaviour and growth rate of the two most common cold-water
17 coral species, *Lophelia pertusa* and *Madrepora oculata*, are affected by micro- or

18 macroplastic exposures. Low-density polyethylene microplastics impair prey capture and

19 growth rates of *L. pertusa* after five months of exposure. Macroplastic films, mimicking

20 plastic bags trapped on deep-sea reefs, had however a limited impact on *L. pertusa* growth.

21 This was due to an avoidance behaviour illustrated by the formation of skeletal 'caps' that

22 changed the polyp orientation and allowed its access to food supply. On the contrary, *M.*

23 *oculata* growth and feeding were not affected by plastic exposure. Such a species-specific

24 response has the potential to induce a severe change in coral community composition and the

25 associated biodiversity in deep-sea environments.

26

27 Growth and feeding behaviour are unchanged for *Madrepora oculata* when exposed to
28 plastics. *Lophelia pertusa* is impacted by microplastics but acclimates to macroplastics.

29

30

31 KEYWORDS

32 Macroplastic litters, microplastics, cold-water corals, biomineralization, *Lophelia pertusa*,
33 *Madrepora oculata*

34

35 INTRODUCTION

36 Anthropogenic activities have a strong negative impact on marine life as extensively
37 documented for metal pollution (Islam and Tanaka, 2004), overfishing (Coll et al., 2008),
38 trawling (Jones, 1992) or ocean acidification (Orr et al., 2005). Plastic pollutants have now
39 been observed in all marine ecosystems (Bergmann et al., 2016; Herrera et al., 2019),
40 including the deep-sea (Woodall et al., 2014). Macroplastics are large debris (> 5 mm of
41 diameter) known to be harmful for marine life (Besseling et al., 2014; Derraik, 2002; Tanaka
42 et al., 2013) and represent the “visible” part of the problem. Microplastics are small particles
43 (< 5 mm; Arthur et al., 2009; GESAMP, 2015) that are formed by the progressive
44 fragmentation of larger plastic debris or directly manufactured as small-size fragments
45 (Rhodes, 2018). Plastic debris can also act as sorption surfaces for hydrophobic organic
46 contaminants more efficiently than sediment particles (Teuten et al., 2007).

47 It has been suggested that animals feed on plastics because they look and smell like prey
48 (Boerger et al., 2010; Fukuoka et al., 2016; Procter et al., 2019; Savoca et al., 2017).

49 Numerous studies have attempted to estimate their impact on marine life (Cole et al., 2015;
50 Hall et al., 2015; Lusher, 2015; Sussarellu et al., 2016) as their toxicity is of concern,

51 including for humans (Wright et al., 2013a). It has indeed been reported that drinking waters
52 of 14 countries from five continents contain microplastic particles (Kosuth et al., 2018),
53 although this assessment is debated (Koelmans et al., 2019). Microplastics can be ingested by
54 zooplankton taxa and transferred to the food web (Setälä et al., 2014), and are also directly
55 ingested by marine fishes (see Herrera et al. 2019 for a recent review), including the ones
56 consumed by humans. Herrera et al. (2019) found for example microplastics in 78.4% of
57 sampled mackerel fish sold on the Canary Islands. The impact of microplastics on health is
58 challenging to assess from *in situ* observations, although some studies have found evidence of
59 tropical corals being contaminated by toxic plastic chemical additives (such as phthalic acid
60 esters; Saliu et al., 2019). Most studies use aquarium experiments and strong negative impacts
61 have been highlighted in a variety of marine taxa (Sussarellu et al., 2016; Tang et al., 2018)
62 including tropical (Hall et al., 2015; Reichert et al., 2018) and deep-sea corals (Chapron et al.,
63 2018). This body of evidence suggests that microplastics represent an equivalent (if not
64 greater) threat than macroplastics to marine communities.

65 As reef-builders in deep-sea environments, scleractinian cold-water corals (CWC) are of
66 paramount importance for sea life as they provide a habitat to biodiversity hotspots compared
67 to adjacent localities deprived of corals (Henry and Roberts, 2007). CWC, such as the colonial
68 species *Lophelia pertusa* (now renamed *Desmophyllum pertusum*; Addamo et al., 2016) and
69 *Madrepora oculata*, form a network of calcium carbonate skeletons consisting in branches of
70 multiple corallites built by individual polyps. Macro- and microplastics have been reported in
71 remote deep-sea coral provinces (La Beur et al., 2019) and macroplastic films have been
72 observed to partially obstruct CWC reefs as they can be trapped in the corallite branches
73 (Angiolillo et al., 2015). In short-term experiments (2.5 months), macroplastics were recently
74 shown to impact the growth and prey capture rates, therefore, effecting colony health and
75 survival of *L. pertusa* (Chapron et al., 2018). Those results, however, were restricted to one

76 species (i.e., *L. pertusa*) and no data is available for long-term exposure. Owing to the
77 difficulties in accessing deep-sea habitats, experiments on CWC are scarce, although it was
78 shown that these deep-sea organisms are exposed both to large plastic wastes and microplastic
79 debris (Taylor et al., 2016). Considering the crucial ecological role of CWC in deep-sea
80 ecosystems and the intensity of plastic pressures in these environments, it is important to
81 evaluate the impact of plastics on CWC in an attempt to forecast the expected changes on
82 coral ecosystems in future oceans.

83 In this study, we present the first investigation at long-term scale (five months) of macro- and
84 microplastic effects on two CWC species (*L. pertusa* and *M. oculata*).

85

86 MATERIALS AND METHODS

87 Origin of colonies

88 Corals used for this study were selected from *L. pertusa* and *M. oculata* colonies collected
89 live from the Lacaze-Duthiers Canyon, in the northwestern Mediterranean Sea (42°32'72"N,
90 03°25'28"E) at 540 m water depth in July 2012 by the Remotely Operated Vehicle (ROV)
91 Super Achille on the R/V Minibex (COMEX Company). Plastic debris have been observed in
92 this location over the years (Fiala-Medioni et al., 2012; Chapron et al., 2018). Sampled corals
93 were transferred onboard to aerated 30 L seawater tanks maintained at a constant temperature
94 of 13°C using a chiller. Once in the laboratory, corals were placed in a dark thermoregulated
95 room at 13°C ± 0.5°C in 80 L tanks receiving continuous flow of 5 µm-filtered Mediterranean
96 Sea water pumped from 5 m water depth. This setting allowed a renewal of over a full tank
97 per day. The colony fragments were each subdivided in nubbins of 3-5 polyps that were
98 subsequently secured on cement blocks using an aquatic epoxy resin (Lartaud et al., 2014). In
99 total, 34 *L. pertusa* and 70 *M. oculata* polyps (from one colony for each species) were used in
100 this study.

101

102 Experimental settings

103 *Aquaria settings*

104 The experimental design is similar to the one published earlier by Chapron et al. (2018). The
105 experiment consisted in three independent semi-closed flumes maintained in the dark at
106 $13\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$ in a thermoregulated room (for microplastics, control and macroplastics
107 exposures; Fig. 1; Supplementary Fig. S1) of 58 L each (Purser et al., 2010). One renewal of
108 seawater per day was set up by a continuous supply of 2.5 L h^{-1} oxygenated, thermoregulated
109 (13°C) and filtered ($5\text{ }\mu\text{m}$) Mediterranean seawater to prevent contamination. A constant flow
110 of 2.5 cm s^{-1} was maintained by a motor (Modelcraft) driven propeller in each flume. A $180\text{ }\mu\text{m}$
111 mesh at the spillway retained microplastic particles within the tank. Corals were
112 acclimated for four weeks in the flumes before plastic addition. During the experiment, corals
113 were fed three times a week with freshly hatched (approx. $500\text{ }\mu\text{m}$ wide) *Artemia salina*
114 nauplii (350 individuals per litre) and once a week with Marine Snow plankton diet (Two
115 Little Fishes Inc, Miami Gardens, USA, 5 mL per flume).

116

117 *Plastic particles*

118 Macro- and microplastics were separately incubated for two months in 5 L seawater tanks
119 continuously supplied with filtered ($20\text{ }\mu\text{m}$) Mediterranean seawater in order to allow
120 bacterial colonization as observed in natural environments (Dussud et al., 2018a).
121 Microplastic beads of low-density polyethylene were added in one flume, with ovoid-shaped
122 beads of the same size ($500\text{ }\mu\text{m}$) and concentration (350 beads L^{-1}) than the ones of the
123 zooplankton used to feed the corals. This ratio of microplastic per zooplankton corresponds to
124 that observed in the Mediterranean surface waters, with concentrations ranging from 0 to 2.28 mg L^{-1}
125 ($\text{Collignon et al., 2012; Pedrotti et al., 2016}$). The quantification of microplastics in the

126 deep-sea has not been reported so far, and measurements are limited by the mesh size of nets
127 used to recover particles (*e.g.*, 333 μm by Dussud et al., 2018b). Moreover, a recent study
128 suggests that the plastic particles content in the deep-sea is largely underestimated (Choy et
129 al., 2019). We therefore chose to use surface water values for the experiment as in Chapron et
130 al. (2018). Macroplastics were constituted of 10 x 10 cm polyethylene film, and added in
131 another flume, directly placed in contact of the cement blocks holding the corals in a way that
132 about 50% of the polyps were obstructed (Fig. 1) as observed in natural settings (Angiolillo et
133 al., 2015). The plastics were approximately 1 cm from the polyps.

134

135 Fig. 1. Experimental design and position of corals related to plastic exposure. The arrow
136 indicates the current direction.

137

138 Labelling and corallite growth measurements

139 At the start of the experiment, the specimens were labelled with fluorescent calcein at 150 mg
140 L^{-1} following the protocol described by Lartaud et al. (2013). The specimens were collected
141 after 5 months of plastic exposure and were cleaned in hydrogen peroxide (4%) at 60°C for 12
142 hours to remove all organic tissues. After embedding in ESCIL SODY 33 epoxy, specimens
143 were cut along the maximum growth axis of corallites using a Buehler Isomet low-speed saw.
144 Sections were polished using alumina down to 0.3 μm . Observations of calcein labels (Fig. 2)
145 were performed using an epifluorescence microscope Olympus BX UCB with an excitation at
146 495 nm (Excelitas X-Cite series 120Q). Distance between the label and the septa apex
147 (corresponding to the death of the coral) was measured (repeated 5 times) using the ImageJ
148 software (<https://imagej.nih.gov/ij/>) and considered as the polyp growth over the experiment
149 (Lartaud et al., 2013; Chapron et al., 2018).

150

151 Fig. 2. Observation of calcein label in a *L. pertusa* septa using an epifluorescence microscope.
152 **a:** Orientation of cut along the maximum growth axis to expose septa of a *L. pertusa*
153 specimen. **b:** Septa exposed once corallite was embedded in resin and cut. The rectangle area
154 indicates the view on **c**. **c:** Fluorescence view of a septa showing the calcein labelling.

155

156 Prey capture rates

157 Coral prey capture rate was measured 92 and 136 days after the start of the experiment
158 following a method described in Purser et al. (2010). Each hour, triplicate 100 mL water
159 samples were collected after feeding. The water samples were filtered (55 µm mesh) and *A.*
160 *salina* nauplii were counted to calculate the concentration of remaining *A. salina* in each
161 flume. The number of zooplankton in each flume was normalized against the number of
162 polyps in the flume, and that number was corrected for the macro- and microplastic flumes
163 against the control flume. Following Purser et al. (2010) and Chapron et al. (2018), results
164 presented focus on the first hour following prey delivery as corals consume over 65% of preys
165 during this period.

166

167 Lipid analyses

168 Nubbins were immediately frozen in liquid nitrogen and stored at -80°C. Samples were then
169 freeze-dried and ground to powder in liquid nitrogen with a TissueLyserII from QIAGEN.
170 Total lipids were extracted from the freeze-dried polyps with chloroform: methanol (2:1) and
171 assayed colorimetrically by the phosphosulfovanillic method (Barnes and Blastock, 1973)
172 using a cholesterol standard. Lipid contents were expressed in mg of cholesterol equivalent
173 and normalized per gram of polyp. Results are given in Supplementary Figure S2.

174

175 Statistical methods

176 Normal distribution for growth rates was checked by Kolmogorov-Smirnov tests on Matlab
177 (v. R2017a). For each test, a normal distribution was rejected at 5% significance level. The *L.*
178 *pertusa* and *M. oculata* populations (growth rates and capture rates) were tested separately to
179 determine if their distributions were identical using Kruskal-Wallis tests at 5% significance
180 level. Multiple comparison procedure by Tukey method was used to determine homogeneity
181 between populations at 5% significance level.

182

183 RESULTS

184 Growth rates

185 No polyp mortality was observed for all conditions during the experiment. Overall, 76% of *L.*
186 *pertusa* corallites and 66% of *M. oculata* corallites exhibited calcein labelling, so that the total
187 number of fragments used for measurements and statistics was 26 and 46 for *L. pertusa* and
188 *M. oculata*, respectively. The measured growth for all septa specimens presenting visible
189 calcein labels are reported in Figure 3. Septal growth for *L. pertusa* was significantly lower in
190 microplastics compared to control conditions (Tukey test, $p = 0.03$), with averages of $307 \pm$
191 $360 \mu\text{m}$ and $1260 \pm 770 \mu\text{m}$ respectively. *Lophelia pertusa* polyps exposed to microplastics
192 also had significantly lower growth than polyps exposed to macroplastic conditions (Tukey
193 test, $p = 0.02$), with a mean growth of $1890 \pm 1670 \mu\text{m}$ for macroplastic conditions. *Lophelia*
194 *pertusa* growth for macroplastics exposure were not statistically different from control
195 settings (Tukey test, $p = 0.95$).

196

197 Fig. 3. Septa growth for *L. pertusa* (left) and *M. oculata* (right) exposed to micro- or
198 macroplastics, or under control conditions at the end of the 5-months experiment. Mean,
199 standard deviation and number of samples are shown. One outlier was observed for *M.*

200 *oculata* in control conditions (indicated by a '+' sign). The letters on top of the boxes indicate
201 significant differences between groups (Tukey tests).

202

203 Contrary to *L. pertusa*, *M. oculata* growth was similar between experimental conditions (Fig.
204 3). Although the mean septal growth was lower under microplastics exposure ($152 \pm 116 \mu\text{m}$,
205 $n=16$) compared to that of macroplastics settings ($189 \pm 159 \mu\text{m}$, $n=15$) and control ($205 \pm$
206 $198 \mu\text{m}$, $n=15$), these values were not statistically different from one another (Kruskal-Wallis
207 test, $p = 0.93$).

208

209 Newly-formed growth structures

210 *Madrepora oculata* did not exhibit any growth abnormalities. However, all but one (86%) *L.*
211 *pertusa* polyps facing the macroplastic films (*i.e.*, with current obstruction) presented a partial
212 cover of the corallite, here called 'caps' (Fig. 4a). The polyp without this overgrowth presents
213 the lowest growth with only $27 \mu\text{m}$ longitudinal growth in 5 months. These 'cap' structures
214 were newly-formed as demonstrated by their position after the calcein labels (Fig. 4b). The
215 'caps' were thinner than the coral wall ($\sim 200 \mu\text{m}$ thick for caps compared to $\sim 1 \text{ mm}$ for coral
216 wall; Fig. 4c). Several millimetres of these 'caps' ($3070 \pm 1090 \mu\text{m}$, $n=4$) were formed during
217 the experiment and were responsible for the highest values of the measured growth rates for
218 corals exposed to macroplastics (Fig. 3). When visible, calcein labels from the wall opposite
219 to that of the 'cap' initiation of these corallites are located at the edge of the septa, indicating
220 that these sides have not grown during the experiment (Fig. 4b and c).

221

222 Fig. 4. A newly-formed 'cap' (growth direction indicated by the red arrow) on *L. pertusa*
223 corallites facing the macroplastic (a). Septa are not concealed by the mineralisation of caps
224 (see septum indicated by the white arrow). Calcein label positioned on a scanning electron

225 microscope view (secondary electron mode, 15 kV) indicates that the ‘caps’ have been
226 formed during the experiment (b). Organization of ‘caps’ on *L. pertusa* corallites relative to
227 the walls in lateral section view (c) indicates that these structures, only present on
228 approximately one-half of the corallite opening, are substantially thin compared to the wall.
229

230 Capture rates

231 Prey capture rates measured after three and five months of plastic exposure are reported in
232 Figure 5. After three months, capture rates for *L. pertusa* exposed to microplastics were not
233 statistically different from those of control specimens (Tukey test, $p = 0.50$; $278 \pm 47 A.$
234 *salina* polyp⁻¹ h⁻¹ and $341 \pm 26 A. salina polyp⁻¹ h⁻¹, respectively). Polyps exposed to
235 macroplastics also exhibited capture rates ($462 \pm 14 A. salina polyp⁻¹ h⁻¹) similar to control
236 specimens (Tukey test, $p = 0.50$), but they were higher than those of microplastic-exposed
237 specimens (Tukey test, $p = 0.03$). After five months of exposure to microplastics, the capture
238 rate of *L. pertusa* had decreased compared to control specimens (Tukey test, $p = 0.05$; $201 \pm$
239 $44 A. salina polyp⁻¹ h⁻¹ and $480 \pm 28 A. salina polyp⁻¹ h⁻¹, respectively). For macroplastics,
240 the capture rates ($462 \pm 28 A. salina polyp⁻¹ h⁻¹) were not significantly different from those of
241 control specimens (Tukey test, $p = 0.73$) but were significantly different from those of
242 microplastic-exposed specimens (Tukey test, $p = 0.05$).$$$$$

243 For *M. oculata*, the capture rates for corals exposed to both micro- ($263 \pm 135 A. salina polyp⁻¹
244 h⁻¹) and macroplastics ($267 \pm 35 A. salina polyp⁻¹ h⁻¹) were similar to control ($269 \pm 25 A.$
245 *salina* polyp⁻¹ h⁻¹) after three months (Tukey test, $p = 0.90$ and $p = 1$, respectively) and five
246 months (Tukey test, $p = 0.98$ and $p = 0.54$, respectively).$$

247

248 Fig. 5. Capture rates of *L. pertusa* (left) and *M. oculata* (right) after 3 months (top) and 5
249 months (bottom) of plastic exposure. Values are normalized against control specimens.
250 Medians and quartiles are indicated.

251

252 DISCUSSION

253 The polyps of *L. pertusa* directly facing macroplastics, and thus more affected by this barrier
254 limiting food supply, developed abnormal growth structures (here called caps) probably
255 aiming at bypassing the plastic obstacle. The new production of caps, observed on all
256 specimens facing the macroplastics, is likely a physiological response that forces the polyp to
257 modify the orientation of its longitudinal growth. This avoidance behaviour will allow the
258 coral to get around the obstacle and reach the nutrient flux again. This growth pattern changed
259 the orientation of the elongation at the expense of wall thickness (Fig. 4c), generating
260 potentially very fragile structures. The absence of growth on the side of the corallites opposite
261 the cap can be explained by a temporary pause of mineralisation, which would resume once
262 the cap is sufficiently strong to support the polyp. This is the first report of such avoidance
263 behaviour at the polyp level. At colony levels, change of direction has been observed in
264 natural settings (Roberts et al., 2009), although only after the full growth of the skeleton, and
265 not at the initiation phase as we suspect happens here. It is also known that sessile taxa other
266 than Scleractinia, such as bivalves, are able to change skeletal or shell growth direction to
267 accommodate for terrain irregularity (Chinzei et al., 1982). This type of coral response to
268 macroplastic exposure was not observed in Chapron et al. (2018) for experiments conducted
269 at shorter timescales (2.5 months). Our results suggest that the cap generation observed here
270 may provide *L. pertusa* successful response to plastic or other obstacles. High lipid contents
271 in the nubbins of *L. pertusa* exposed to macroplastics confirm their good fitness
272 (Supplementary Fig. S2) and suggest that the production of cap represents for the colony an

273 efficient strategy to overcome the obstructive effect of macroplastics or other obstacles. The
274 reason why this response was however not observed for shorter exposure time (Chapron et al.,
275 2018) is yet unknown.

276 Our study also reports a species-specific impact of plastic exposure for CWC for both micro-
277 and macroplastics. While *L. pertusa* showed close similar patterns than those described in
278 Chapron et al. (2018) *M. oculata* skeletal growth and capture rates were not impaired by
279 microplastics exposure. One reason may be that *M. oculata* does not effectively capture the
280 microplastics in the size range used in the experiment. *Madrepora oculata* could be selective
281 in its diet and/or could display rejection behaviour for preys that have similar size to *Artemia*
282 nauplii, which were used in our experiment. Indeed, it was observed that different taxa ingest
283 different size of microplastic beads (van Cauwenberghe et al., 2015). This is supported by
284 recent unpublished observations highlighting that *M. oculata* stores more energy when fed on
285 phytoplankton rather than *Artemia* nauplii (Galand et al., submitted), and captures more
286 effectively large *Artemia* (*i.e.*, 1000 μm) than nauplii (500 μm) (Surhoff et al., submitted).
287 Inversely, it has been reported that *L. pertusa* is opportunistic in terms of diet, which ranges
288 from phytodetritus (van Oevelen et al., 2009) to zooplankton (Freiwald, 2002; Kiriakoulakis
289 et al., 2005) with prey size reaching 10 mm (Tsounis et al., 2010). If prey size selection is at
290 play, *M. oculata* may exhibit equivalent negative impact than that observed on *L. pertusa* here
291 when exposed to particles of different size than 500 μm .

292 The growth and capture rates of *M. oculata* were also unaffected by macroplastic exposure.
293 *Madrepora oculata* polyps are smaller than those of *L. pertusa* (Lartaud et al., 2017). The
294 macronutrient flux, although limited by the macroplastic obstacle, may be sufficient to
295 provide the small polyps with food. Alternatively, the *M. oculata* polyps facing away from the
296 obstacle could share their nutritional resources and/or energy with the other polyps in a more
297 efficient way than does *L. pertusa*. Colonial organisms have been shown to share their

298 proteins between adjacent and sometimes remote polyps (Buss et al., 2015). However, *M.*
299 *oculata* polyps, contrary to *L. pertusa*, do not exhibit a physical link at the polyp basis, inside
300 the skeleton (Lartaud et al., *in press*), which opens additional questions regarding the
301 pathways of resource and energy sharing between polyps of a same branch or colony.
302 Species-specific vulnerability to plastic pollutants has previously been reported on tropical
303 coral species (Reichert et al., 2018). In their study, Reichert et al. (2018) showed that six coral
304 species exhibit different responses when exposed to microplastics for four weeks, such as
305 attachment to tentacles, mucus production, overgrowth and ingestion of microbeads. From the
306 six studied species, five exhibited negative health impacts, with the only species visibly not
307 impacted being *Porites lutea*, which was the only species using mucus production against the
308 exposure. In our study, mucus production was observed from both *L. pertusa* and *M. oculata*,
309 as it is often observed for cold-water coral species. This strategy seems ineffective for *L.*
310 *pertusa*.

311 After 5 months of exposure, *L. pertusa* exhibited significantly reduced growth rates when
312 exposed to microplastics. The energy balance plays a key role in the species' potential for
313 survival and acclimation to stress. In general, when conditions are optimal, energy is stored or
314 allocated for growth and reproduction, but reserves are depleted to withstand the additional
315 costs of stressful conditions (Lesser, 2013; Rossi and Tsounis, 2007). Therefore, our
316 measurements of lipids (Supplementary Fig. S2) indicate that control specimens presented
317 low energy reserves likely due to allocation to skeletal growth, while microplastic-exposed
318 polyps showed similar reserves without growth. Those results are in accordance with a short-
319 term (2.5 months) experiment showing a significant impact of plastics on growth of *L. pertusa*
320 (Chapron et al., 2018). The authors suggested a decrease of energy storage due to the
321 ingestion and egestion costs of microplastic beads. This is in agreement with observations
322 from shallow-water coral species that egested most of plastic particles within 24 to 48 h after

323 ingestion, but that energy supply (through consumption) and thus growth was expected to be
324 reduced (Allen et al., 2017; Hankins et al., 2018). This reduction in capture rates has also been
325 shown on the polychaete worm for which a chronic microplastic exposure inhibited feeding
326 activity, which in turn reduced energy storage and organism fitness (growth rates and
327 survival; Wright et al. 2013b). This could be due to a potential simulation of satiation or
328 blockages of digestive cavities by microplastics as suggested in crustaceans and fishes (Cole
329 et al., 2015; Critchell and Hoogenboom, 2018; Murray and Cowie, 2011; Watts et al., 2015).
330 Interestingly, Chapron et al. (2018) reported that after 2.5 months of exposure capture rates
331 reached those of the control specimens. Here, we also observed that capture rates in
332 microplastic and control settings were not significantly different from each other after 3
333 months of exposure. However, an extended period of exposure to microplastics (5 months)
334 had a significant negative impact on capture rate. A possible explanation is that *L. pertusa*
335 compensates for microplastic exposure during several weeks but eventually fails to reach a
336 sustainable state under this stress, hence dramatically reducing its feeding behaviour. Growth
337 rate reaching normal values under microplastic exposure is therefore unlikely over a longer
338 period (*i.e.*, over five months), and lingering negative impacts are to be expected.

339

340 CONCLUSION

341 This paper reports for the first time the effects of long-term micro- and macroplastic exposure
342 on two cold-water coral species, *L. pertusa* and *M. oculata*. *Lophelia pertusa* was greatly
343 affected by microplastic beads, both in terms of prey capture and growth rates. The impact of
344 water current obstruction by macroplastic films appears limited as it was bypassed by polyp
345 skeleton overgrows aiming at getting around the plastic obstacles. On the contrary, *M. oculata*
346 was not impacted in either condition, as no difference was observed between control, micro-
347 and macroplastic settings. We conclude that plastic pollution in nature, by impacting one

348 species more than another, could lead to a decrease in biodiversity in cold-water coral
349 ecosystems. Additional impacts on the associated fauna may also occur, as *L. pertusa* and *M.*
350 *oculata* are generally associated to improve the coherence of the carbonate reefs and protect
351 other taxa.

352

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361

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Figure captions:

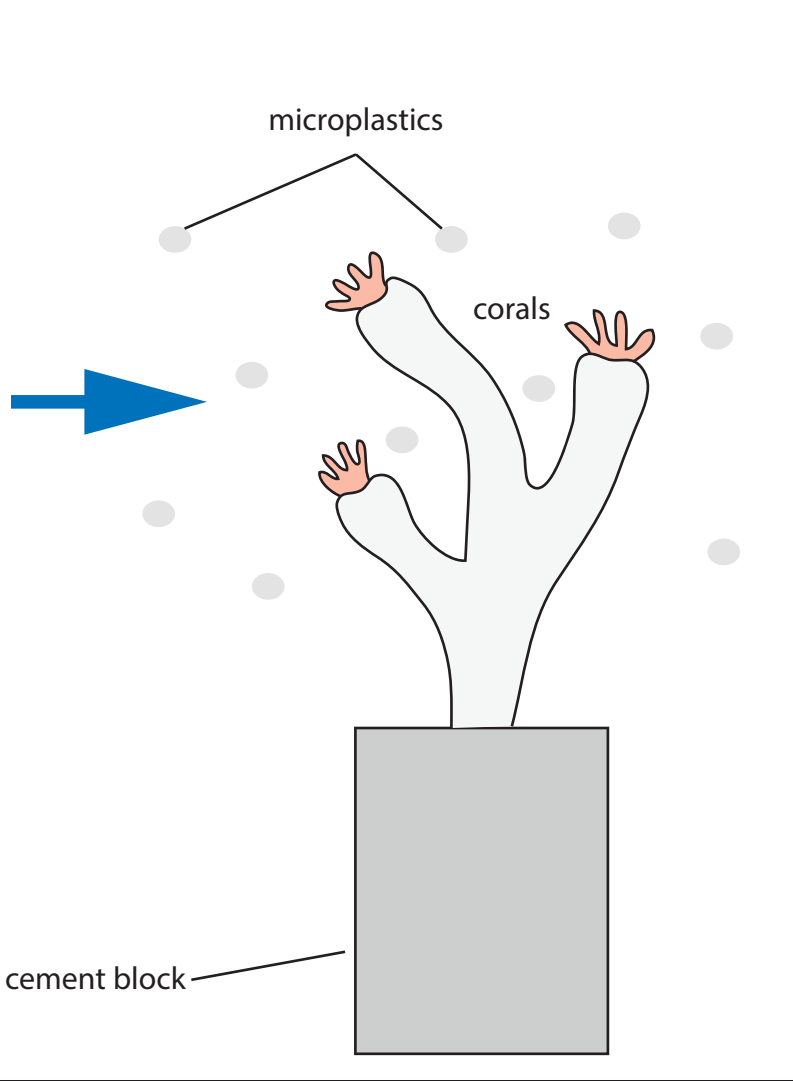
Fig. 1. Experimental design and position of corals related to plastic exposure. The arrow indicates the current direction.

Fig. 2. Observation of calcein label in a *L. pertusa* septa using an epifluorescence microscope. **a:** Orientation of cut along the maximum growth axis to expose septa of a *L. pertusa* specimen. **b:** Septa exposed once corallite was embedded in resin and cut. The rectangle area indicates the view on **c**. **c:** Fluorescence view of a septa showing the calcein labelling.

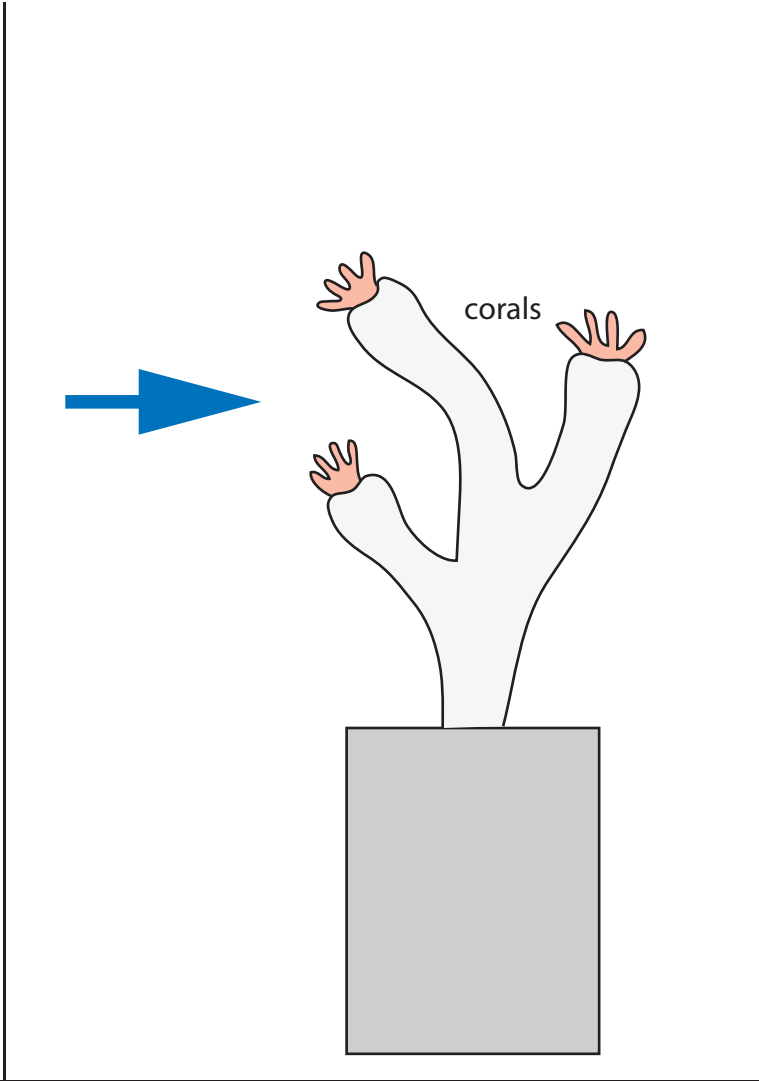
Fig. 3. Septa growth for *L. pertusa* (left) and *M. oculata* (right) exposed to micro- or macroplastics, or under control conditions at the end of the 5-months experiment. Mean, standard deviation and number of samples are shown. One outlier was observed for *M. oculata* in control conditions (indicated by a '+' sign). The letters on top of the boxes indicate significant differences between groups (Tukey tests).

Fig. 4. A newly-formed 'cap' (growth direction indicated by the red arrow) on *L. pertusa* corallites facing the macroplastic (**a**). Septa are not concealed by the mineralisation of caps (see septum indicated by the white arrow). Calcein label positioned on a scanning electron microscope view (secondary electron mode, 15 kV) indicates that the 'caps' have been formed during the experiment (**b**). Organization of 'caps' on *L. pertusa* corallites relative to the walls in lateral section view (**c**) indicates that these structures, only present on approximately one-half of the corallite opening, are substantially thin compared to the wall.

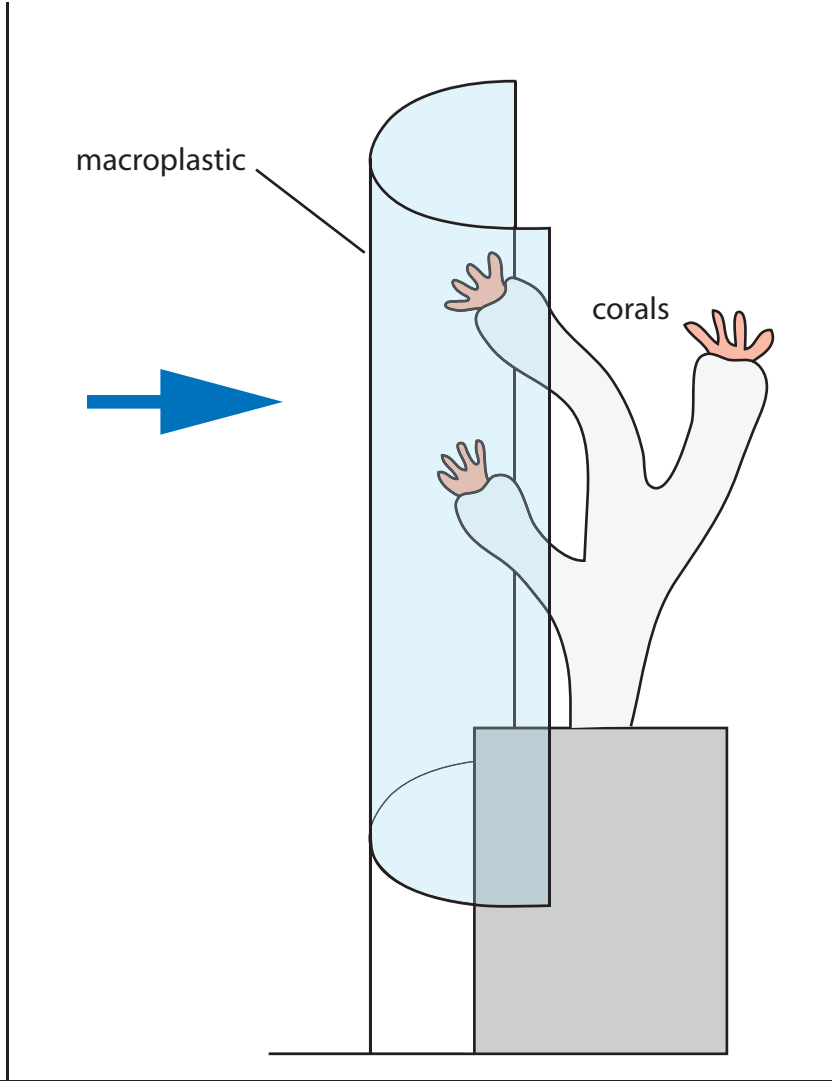
Fig. 5. Capture rates of *L. pertusa* (left) and *M. oculata* (right) after 3 months (top) and 5 months (bottom) of plastic exposure. Values are normalized against control specimens. Medians and quartiles are indicated.



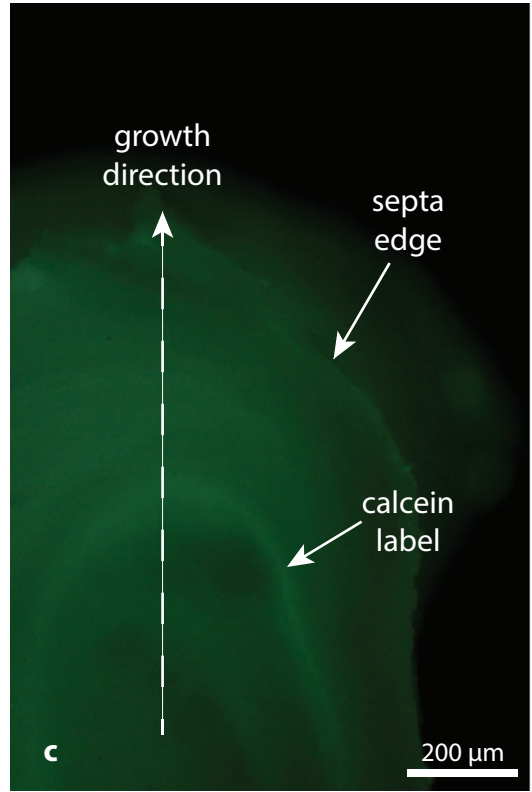
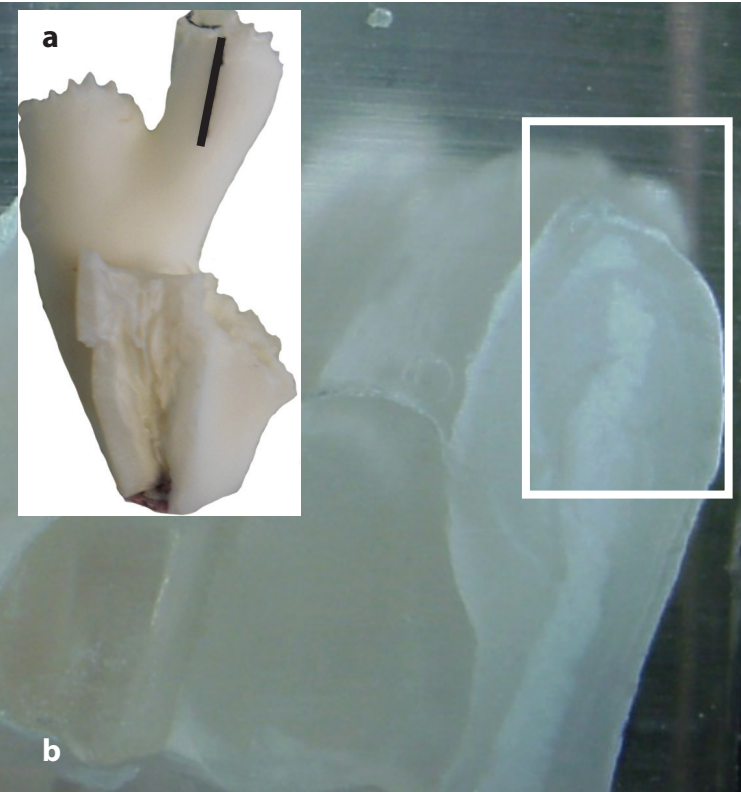
Microplastics settings



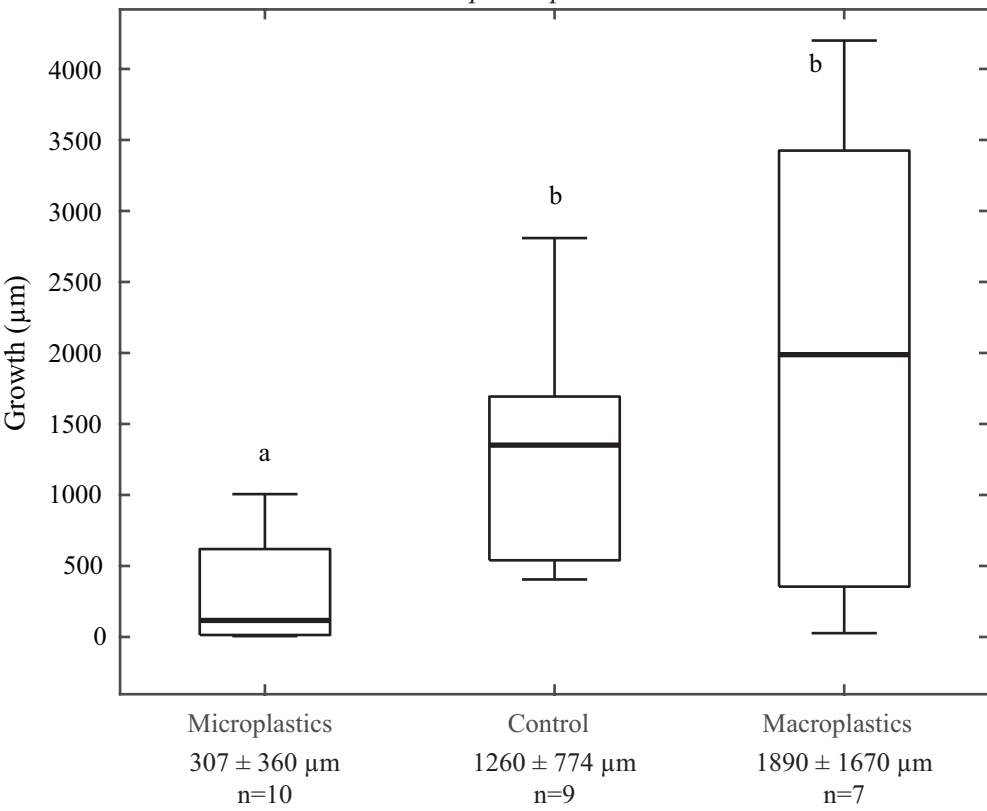
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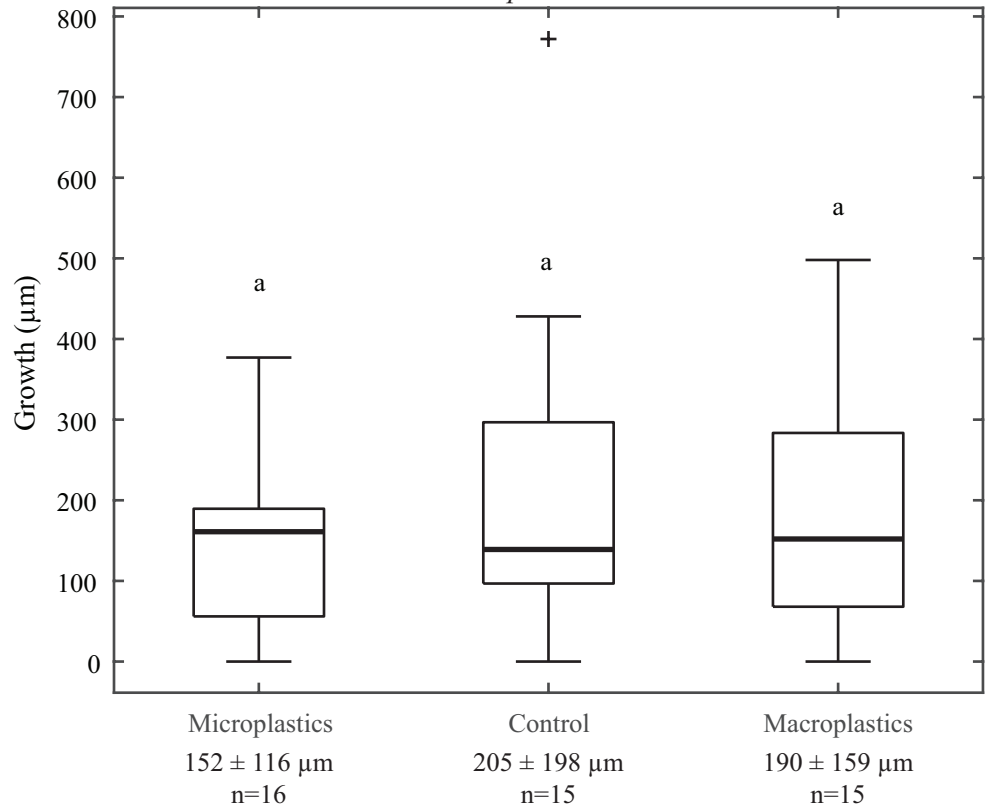
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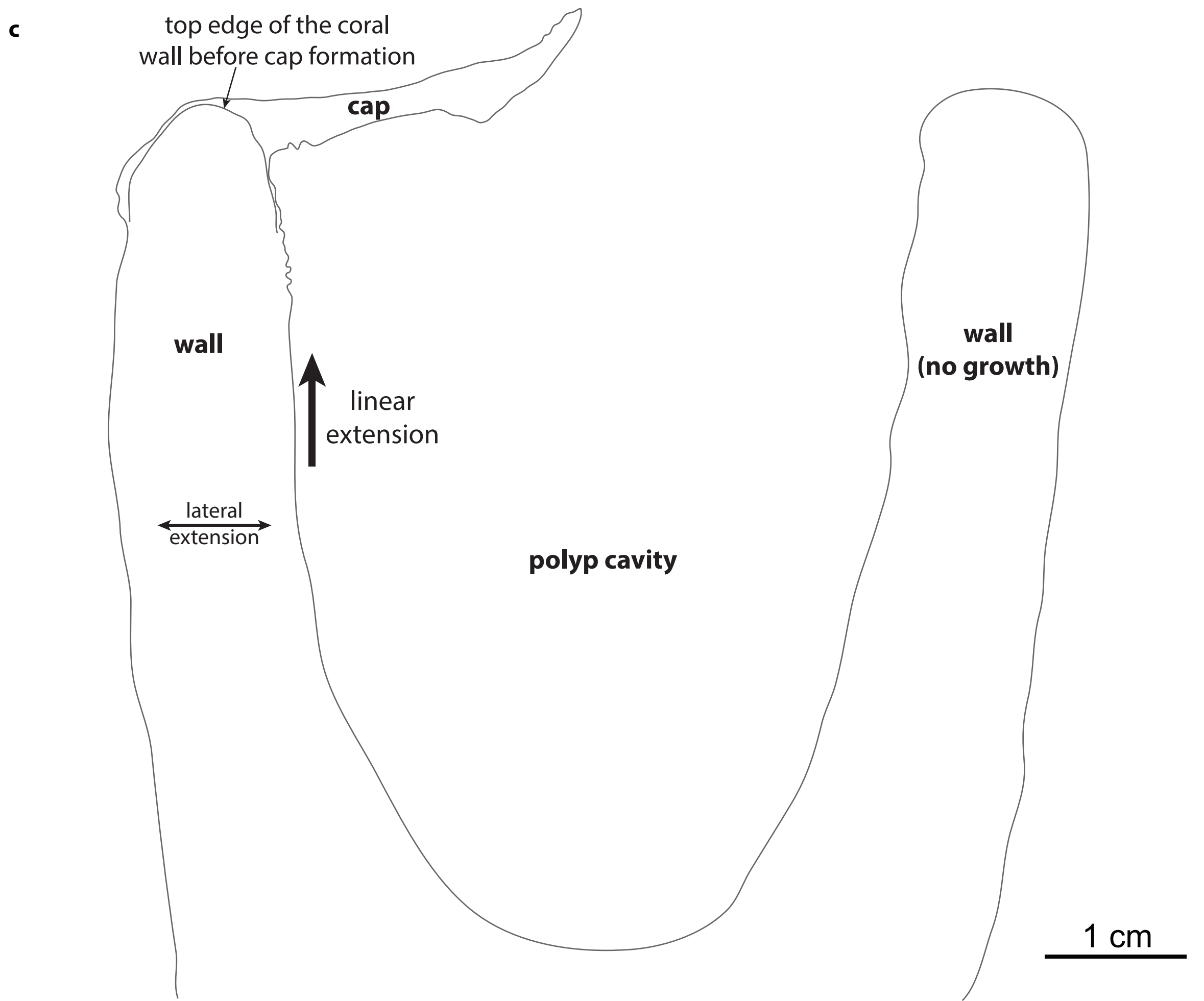
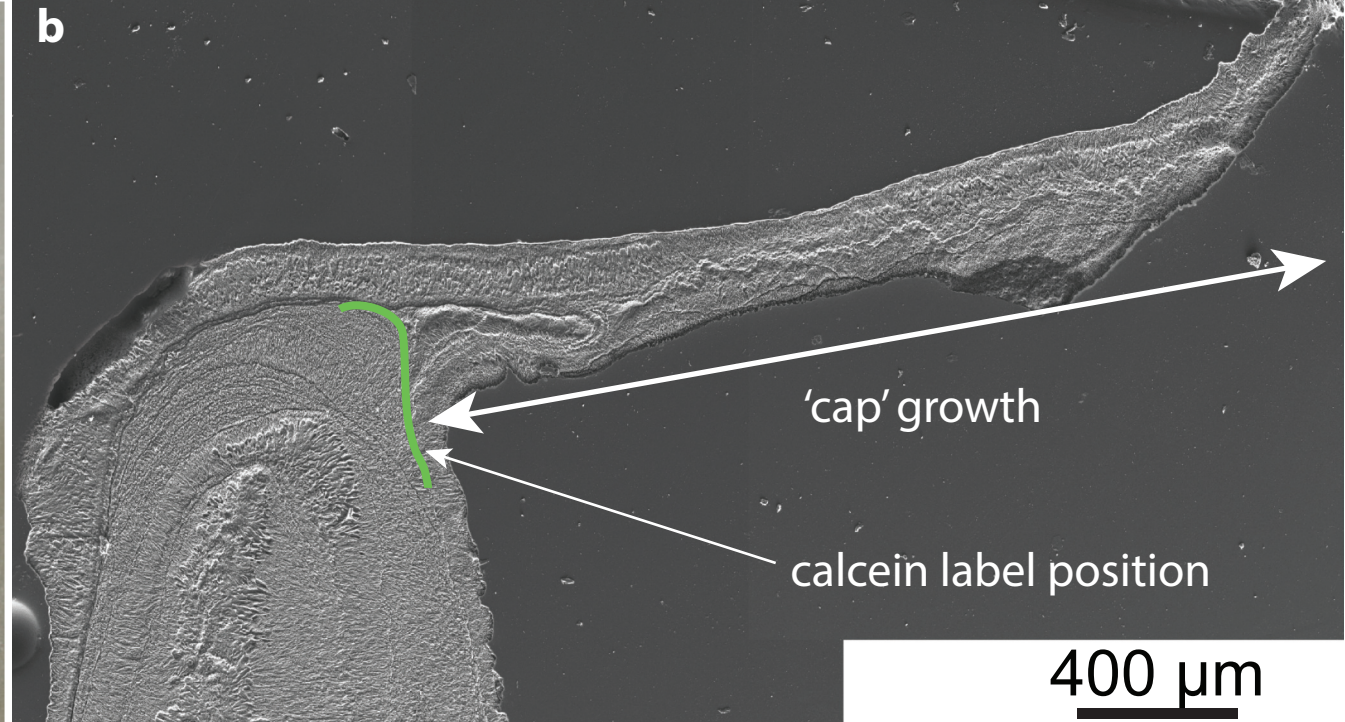
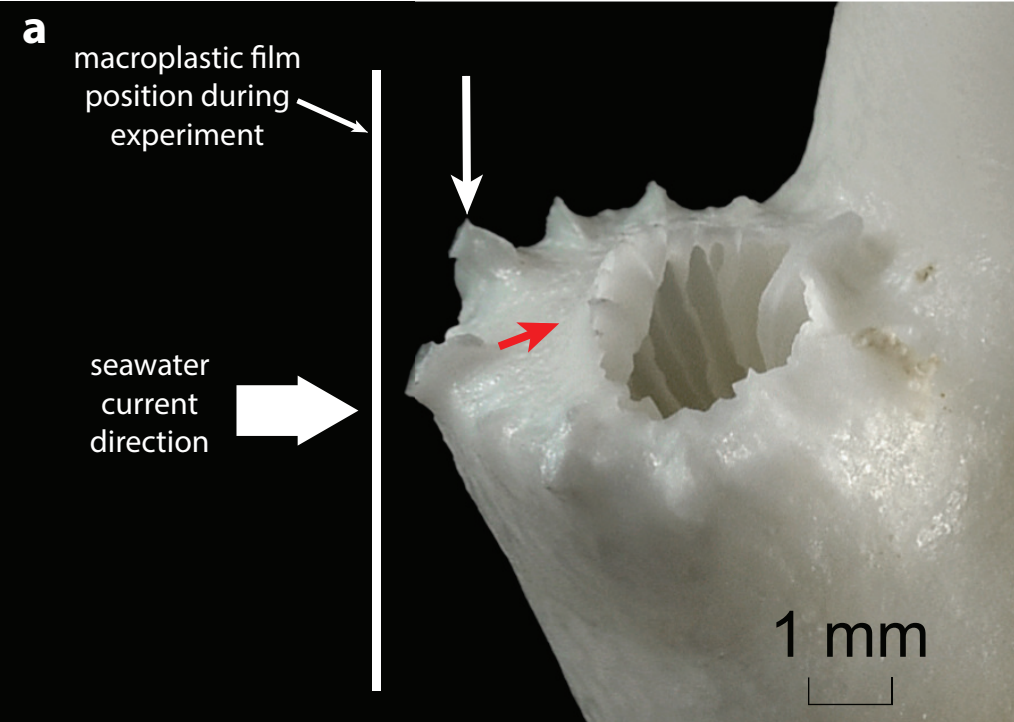


Lophelia pertusa



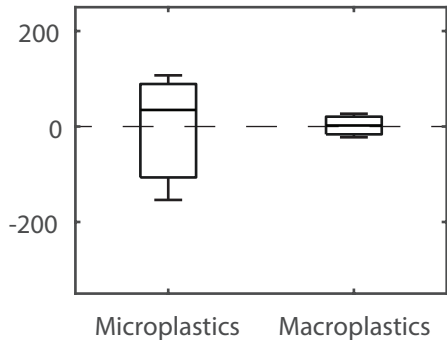
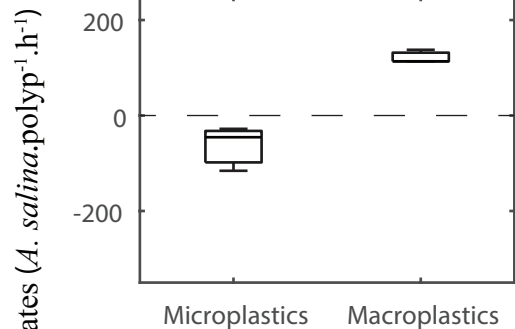
Madrepora oculata



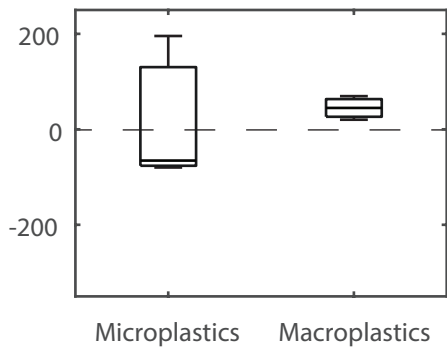
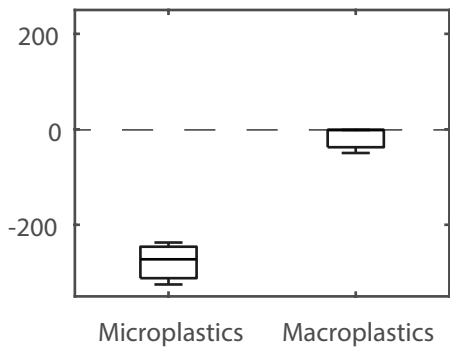


Lophelia pertusa

Madrepora oculata



t = 3 months



t = 5 months

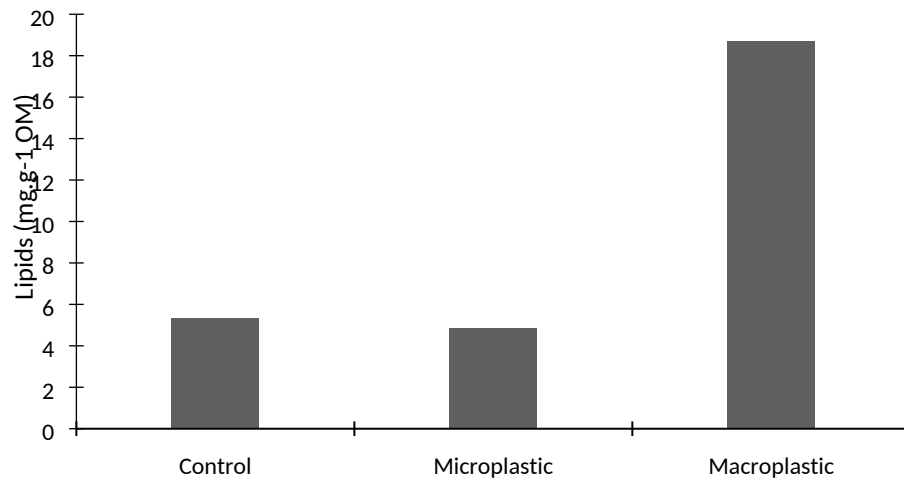
Supplementary Figure S1 of Mouchi et al.: "Long-term aquaria study suggests species-specific responses of two cold-water corals to macro- and microplastics exposure".



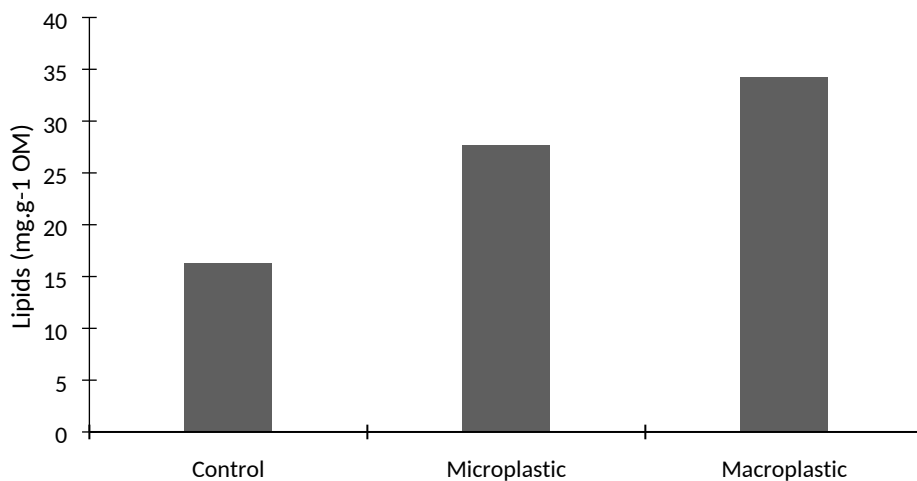
Supplementary Fig. S1: Corals in aquaria with macroplastic films (a) and microplastic beads (b).

Supplementary Figure S2 of Mouchi et al.: “Long-term aquaria study suggests species-specific responses of two cold-water corals to macro- and microplastics exposure”.

a- *Lophelia pertusa*



b- *Madrepora oculata*



Supplementary Figure S2: Lipid concentration in coral nubbies of *Lophelia pertusa* (a) and *Madrepora oculata* (b) after 5 months of exposure to control conditions, microplastics and macroplastics. Polyps from several nubbies were pooled for the analyses. Lipids are expressed in mg of equivalent cholesterol per gram of organic matter. The analytic error for the measure is less than 10%.